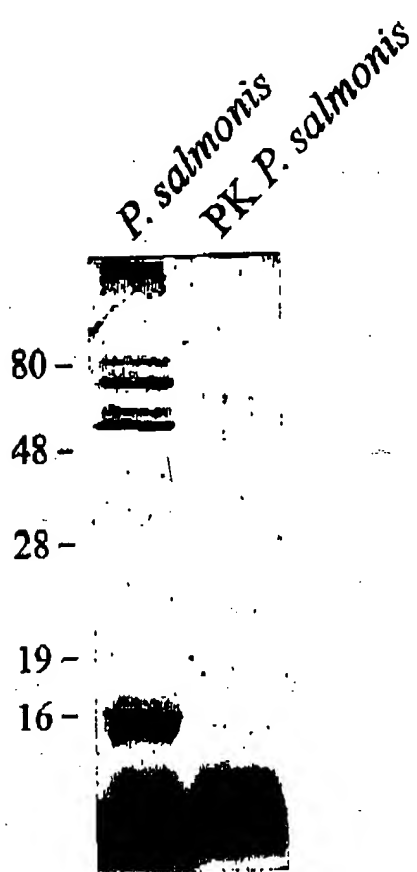


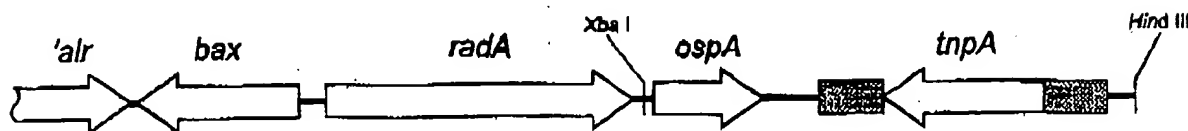
FIGURE 1. WESTERN BLOT ANALYSIS OF *P. SALMONIS*



005734-051500

FIGURE 2

A. ORF's in the region of the *ospA* gene from *P. salmonis*



B. DNA sequence of *ospA* gene from *P. salmonis* (SEQ ID:1)

ATGAACAGAGGATGTTTGCAGGTAGTAGTCTAATTATTATCAGTGTGTTTTTGTAGTTGGCTGTGCCCAGA
 ACTTTAGTCGTCAAGAAGTCGGAGCTGCGACTGGGGCTGTTGTTGGCCGGTGTGCTGGCCAGCTGTTTGG
 TAAAGGTAGTGGTCGAGTTGCAATGGCCATTGGTGGTGCTGTTTTGGGTGGATTAAATTGGTTCTAAAATC
 GGTCAATCGATGGATCAGCAGGATAAAATAAAGCTAAACCAGAGTTTGGAAAAGGTAAAAGCAGGGCAAG
 TGACACGTTGGCGTAATCCAGATACAGGCAATAGTTATAGTGTGAGCCAGTGCCTACTTACCAGCGTTA
 CAATAAGCAAGAGCGTCGCCAGCAATATTGTCGAGAATTTAGCAAAAAGGCGATGATTGCAGGGCAGAAG
 CAAGAGATTTACGGCACTGCATGCCCGCAACCGGATGGTCGTTGGCAAGTCATTTCAACAGAAAAA

Amino acid sequence of OspA protein (SEQ ID:2)

MNRGCLQGSSLIISVFLVGCQNFSRQEVGAATGAVVGGVAGQLFGKSGSRVAMAIGGAVLGGLIGSKI
 GQSMDDQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQCYCREFOOKAMIAGQK
 QEIYGTACROPDGRWQVISTEK

C. Sequence alignment of the OspA proteins of *P. salmonis* and *R. prowazekii*

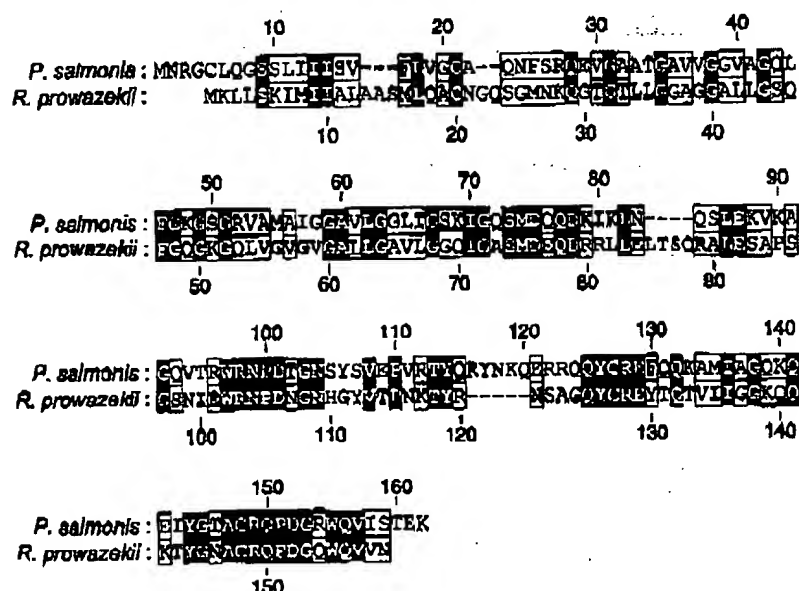
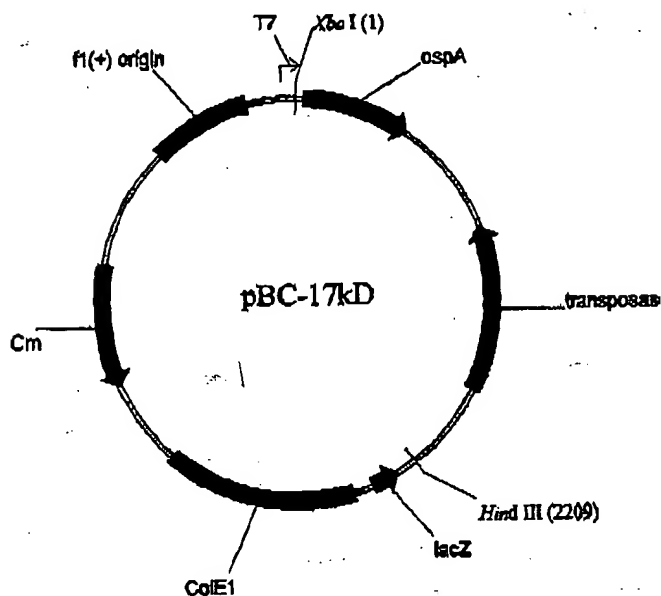


FIGURE 3

A. Map of plasmid pBC-17kDa encoding the *ospA* ORF.



B. Western blot analysis of OspA expression.

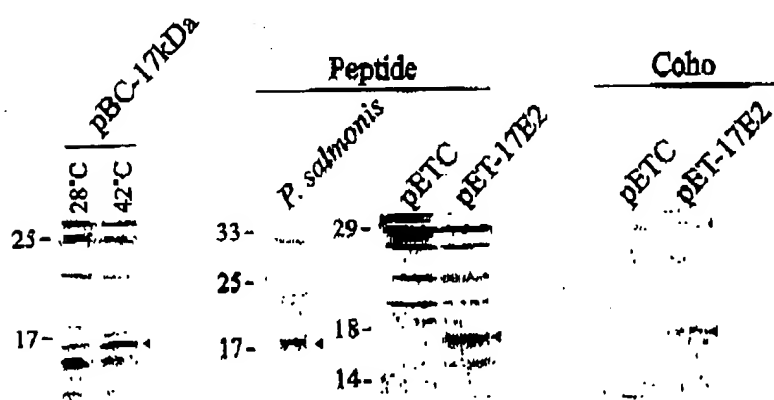
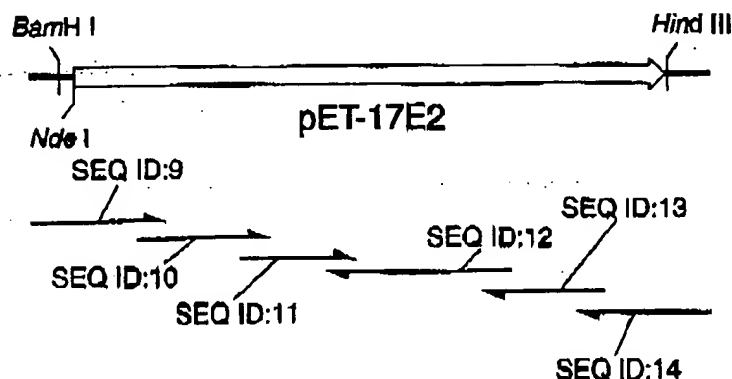


FIGURE 4.

A. Strategy for construction of the *E. coli* codon optimized *ospA* gene.



B. Oligonucleotide #1 (SEQ ID:9)

CGCCAGGGTTTTCCAGTCACGACGGATCCGTCTCATATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATC
ATTATCTCTGTTTTCTGGTGGGTTGCGCCAGAACTTCAG

Oligonucleotide #2 (SEQ ID:10)

TGGGTTGCGCCAGAACTTCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGC
CGCCAGCTGTTGGTAAAGGCTCTGGTCGTGTGGCGATG

Oligonucleotide #3 (SEQ ID:11)

AAAGGCTCTGGTCGTGTGGCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAATCG
GTCAGAGCATGGACCAGCAGGATA

Oligonucleotide #4 (SEQ ID:12)

GTTCCACAGAGTAGCTGTTACCGGTGTCCGGATTACGCCAACGAGTAACCTGGCCGGCTTTCACTTTTTC
CAGAGACTGGTTCAGTTTTGATTTTATCCTGCTGGTCCATGCTCTGACC

Oligonucleotide #5 (SEQ ID:13)

GGTGCCGTAGATTTCTGTTTTCTGACCTGCGATCATGGCTTTCTGCTGAAAATTCGCGGCAGTACTGCTGA
CGGCGTTTCTGTTTGTGTAACGCTGGTAGGT

Oligonucleotide #6 (SEQ ID:14)

CGTCCTCTCGTCCTGGTCCGAATTCAGATAAGCTTATTTTTCGGTGCTAATCACCTGCCAGCGGCCATCC
GGCTGACGGCACGCGGTGCCGTAGATTTCTGTTTTCTGAC

C. DNA sequence of *E. coli* optimized *ospA* gene, 17e2 (SEQ ID:3)

ATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATCATTATCTCTGTTTTCTGGTGGGTTGCGCCAGAACT
TCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGCGGCCAGCTGTTCCGTAA
AGGCTCTGGTCGTGTGTCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAATCGGT
CAGAGCATGGACCAGCAGGATAAAATCAAACCTGAACAGTCTCTGGAAGAAAGTGAAGCCGCCAGGTTA
CTCGTTGGCGTAATCCGGACACCGGTAACAGTACTCTGTGGAACCGGTTCCGACCTACCAGCGTTACAA
CAAACAGGAACGCCGTGACGAGTACTGCCCGGAATTCAGCAGAAAGCCATGATCGCAGGTGAGAAACAG
GAAATCTACGGCACCGCGTGCCCTCAGCCGGATGGCCGCTGGCAGGTGATTAGCACCGAAAAA

FIGURE 5

A. Amino acid sequence of optimized OspA protein, 17E2, (SEQ ID:4).

MRGCLQGSSLIISVFLVGCAQNFSRQEVGAATGAVVGGVAGQLFGKGSGRVSMAGGAVLGGLIGSKIG
QSMDOQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEFVRTYQRYNKQERRQOYCREFOQKAMIAGOKQ
EIIYGTACPQPDGRWQVISTEK

B. DNA sequence of c17e2 *ospA* construct with N-terminal fusion partner (SEQ ID:5).

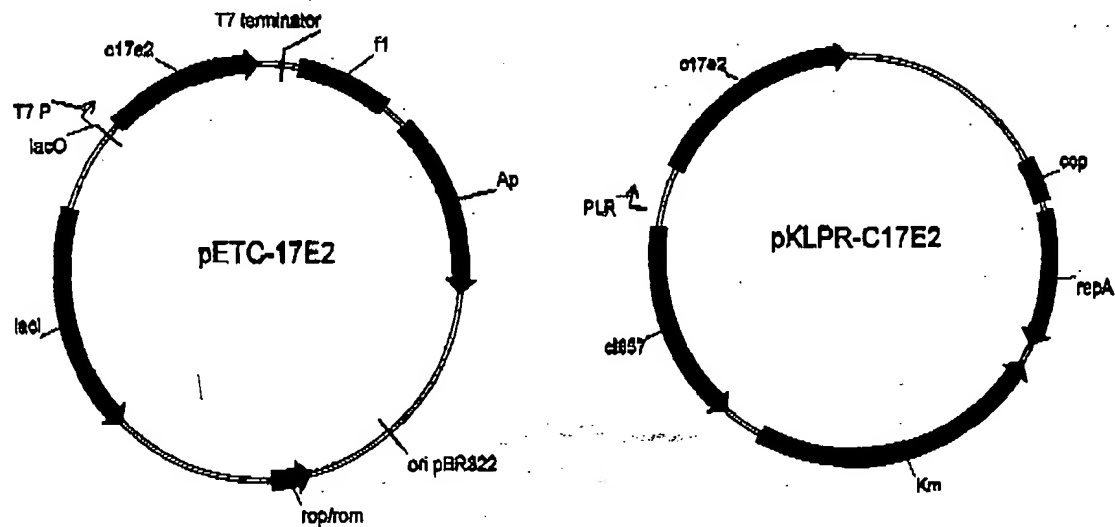
ATGTCAGTTGAATTCTACAACCTCTAACAAATCAGCACAAACAACTCAATTACACCAATAATCAAAATTA
CTAACACATCTGACAGTGATTTAAATTTAAATGACGTAAAAGTTAGATATTATTACACAGTGATGGTAC
ACAAGGACAAACTTTCTGGTGTGACCATGCTGGTGCATTATTAGGAAATAGCTATGTTGATAACACTAGC
AAAGTGACAGCAAACTTCGTTAAAGAAACAGCAAGCCCAACATCAACCTATGATACATATCTGGATCCGT
CTCATATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATCATTATCTCTGTTTTCTCGTGGGTTGCGCCCA
GAACTTCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGCCGCCAGCTGTTT
GGTAAAGGCTCTGGTCTGTGTGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAA
TCGGTCAGAGCATGGACCAGCAGGATAAAATCAAACCTGAACAGTCTCTGAAAAAGTGAAGCCGGCCA
GGTTACTCGTTGGCGTAATCCGGACACCGGTAACAGCTACTCTGTGGAACCGGTTGCGACCTACCAGCGT
TACAACAAACAGGAACGCCGTGAGCAGTACTGCCGCGAATTTGAGCAGAAAGCCATGATCGCAGGTGAGA
AACAGGAAATCTACGGCACCGCGTGCCCTCAGCCGGATGGCCGCTGGCAGGTGATTAGCACCGAAAAA

C. Amino acid sequence of C17E2 OspA construct with N-terminal fusion partner (SEQ ID:6).

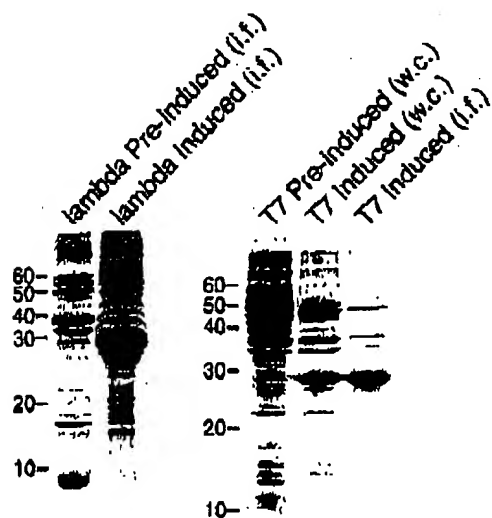
MSVEFYNSNKS AQTNSITPIIKITNTSDSDLNLNDVKVRYTSDGTGGQTFWCDHAGALLGNSYVDNTS
KVTANFVKETASPTSTYDTYLDPSHMRGCLQGSSLIISVFLVGCAQNFSRQEVGAATGAVVGGVAGQLF
GKGSGRVSMAGGAVLGGLIGSKIGQSMDOQDKIKLNQSLKVKAGQVTRWRNPDTGNSYSVEFVRTYQRY
YNKQERRQOYCREFOQKAMIAGOKQEIIYGTACPQPDGRWQVISTEK

FIGURE 6

A. Expression vectors encoding the optimized *ospA* fusion constructs



B. SDS-PAGE analysis of C17E2 expression.



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FIGURE 7

Map of the *ospA*-fusion construct encoding a C-terminal fusion partner under T7 promoter control.

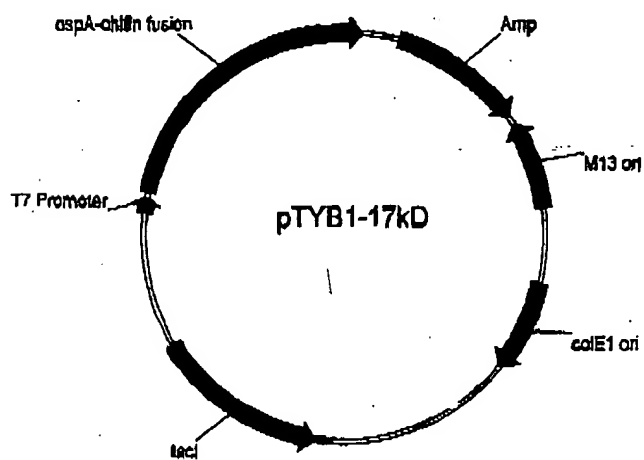
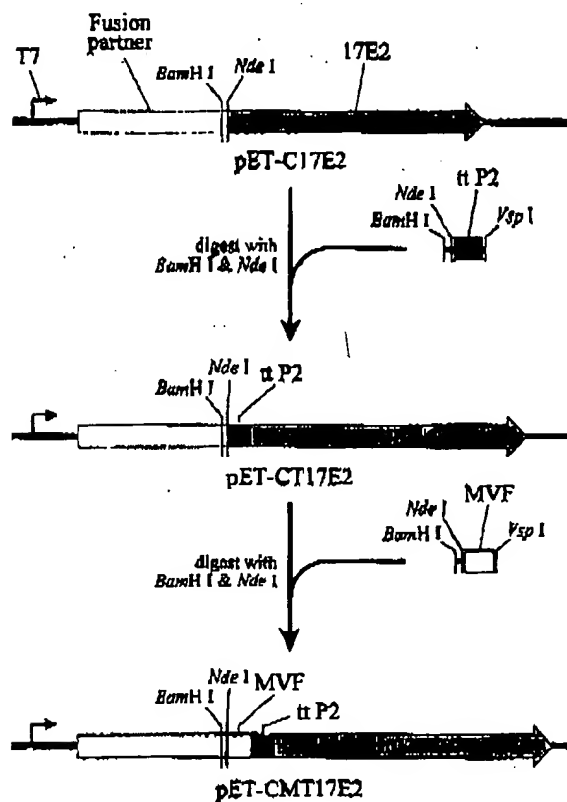


FIGURE 8

A. CLONING STRATEGY FOR OSPA TCE FUSION PROTEIN CONSTRUCTS.



B. (a) Nucleotide sequence of the *tt* P2 oligonucleotide (SEQ ID:17)

CGCCAGGGTTTTCCCAGTCACGACGGATCCGTCTCATATGCAGTACATTAAAGCAAACCTCTAAATTCATC
GGTATTACCGAACTGATTAATTAAGCTTCGGACCAGGACGAGAGGACG

(b) Nucleotide sequence of the MVF oligonucleotide (SEQ ID:18)

CGCCAGGGTTTTCCCAGTCACGACGGATCCGTCTCATATGCTGTCTGAAATCAAAGGTGTTATCGTTCAT
CGTCTGGAAGGCCTGATTAATTAAGCTTCGGACCAGGACGAGAGGACG

(c) Amino acid sequence of the *tt* P2 TCE (SEQ ID:19)

QYIKANSKFIGITEL

(d) Amino acid sequence of the MVF TCE (SEQ ID:20)

LSEIKGVTVHRLEGV

FIGURE 9

Coho salmon antibody titres against OspA-fusion protein candidate vaccines.

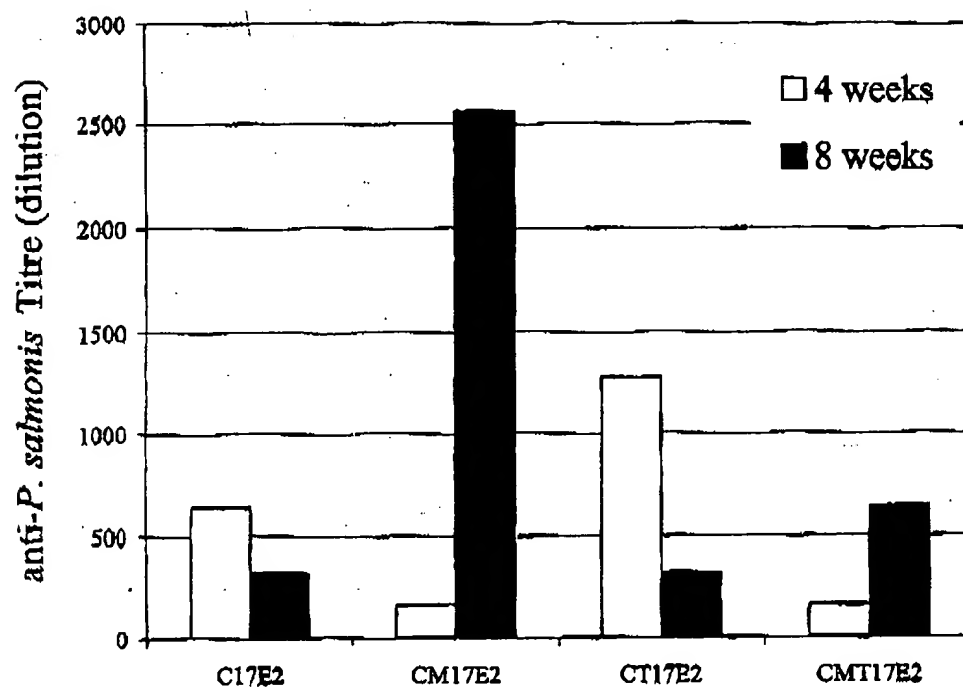


FIGURE 10

Whole lymphocyte proliferative response to OspA-fusion proteins in Atlantic salmon.

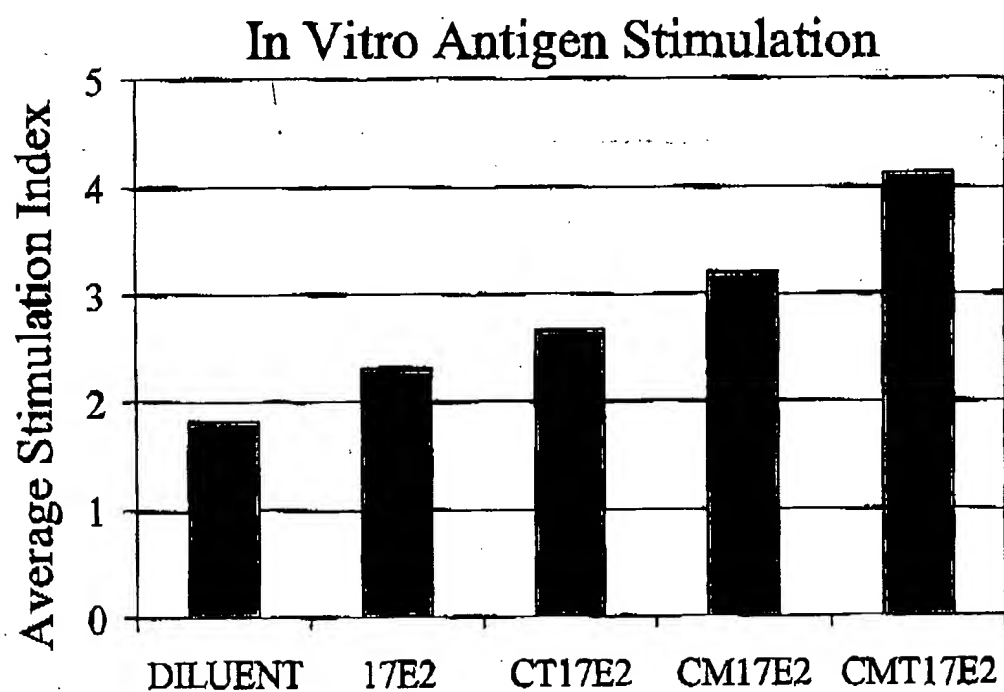


FIGURE 11

Vaccine trial in coho salmon of OspA fusion proteins.

